# NEEDLE-FREE INHALABLE VACCINE AND ANTIBIOTIC POWDER AEROSOLS

**R. E. Sievers, J.L. Burger,, S. P. Cape, E.T.S. Huang, J.A. Best, J.A. Madsen and L.Rebits**

**Center for Pharmaceutical Biotechnology,**
**Dept. of Chemistry and Biochemistry, and CIRES,**
**214 UCB, University of Colorado, Boulder, CO 80309**
**and AKTIV-DRY,**
**6060 Spine Road, Boulder, CO 80301**

# Report Documentation Page

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
|---|---|---|
| **15 NOV 2004** | **N/A** | **-** |

| 4. TITLE AND SUBTITLE | 5a. CONTRACT NUMBER |
|---|---|
| **Needle-Free Inhalable Vaccine And Antibiotic Powder Aerosols** | 5b. GRANT NUMBER |
| | 5c. PROGRAM ELEMENT NUMBER |
| 6. AUTHOR(S) | 5d. PROJECT NUMBER |
| | 5e. TASK NUMBER |
| | 5f. WORK UNIT NUMBER |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Center for Pharmaceutical Biotechnology, Dept. of Chemistry and Biochemistry, and CIRES, 214 UCB, University of Colorado, Boulder, CO 80309** | 8. PERFORMING ORGANIZATION REPORT NUMBER |
| 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) | 10. SPONSOR/MONITOR'S ACRONYM(S) |
| | 11. SPONSOR/MONITOR'S REPORT NUMBER(S) |

12. DISTRIBUTION/AVAILABILITY STATEMENT
**Approved for public release, distribution unlimited**

13. SUPPLEMENTARY NOTES
**See also ADM001849, 2004 Scientific Conference on Chemical and Biological Defense Research. Held in Hunt Valley, Maryland on 15-17 November 2004 . , The original document contains color images.**

14. ABSTRACT

15. SUBJECT TERMS

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | |
| **unclassified** | **unclassified** | **unclassified** | **UU** | **29** | |

# ROOM-TEMPERATURE-STABLE VACCINES, ENZYMES, ANTIBODIES, ANTIBIOTICS, AND PHARMACEUTICALS

**Strategy: Stabilize and dry powders near room temperature by supercritical or near-critical fluid carbon dioxide processing**

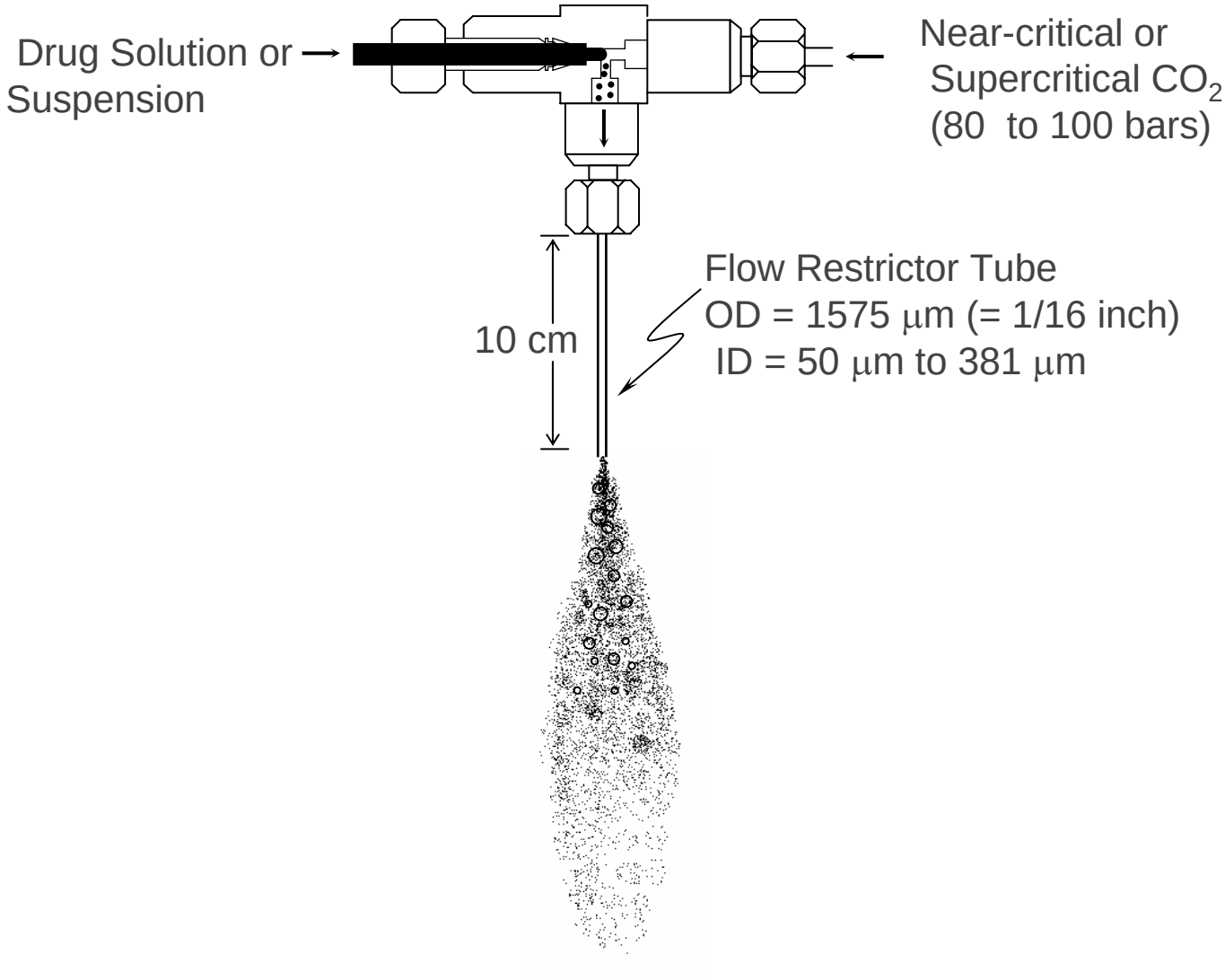
- **Needle-free delivery of respirable vaccines (e.g., measles), antibiotics, and pharmaceuticals**
- **Nanoparticle and microparticle synthesis and coating**
- **Micronize dry powder antibiotics, enzymes, antibodies, e.g., Cipro Moxifloxacin, rifampicin, antitrypsin, IgG, for delivery into**
- **lungs (1-3 microns), or into nasal passages (10-30 microns)**
- **Stabilize in glassy sugar matrices, then micronize and desolvate**
  - **-Avoid aggregation and create rapidly redissolved microparticles**
  - **-Enhance bioavailability by increasing surface areas of particles, e.g., with smaller particles or leached composite particles**

# The Principle of a New CAN-BD Process: (Carbon Dioxide Assisted Nebulization with a Bubble Dryer®)

- In CAN-BD a solution or suspension in water (or, when necessary, an organic solvent) is mixed intimately in a low volume tee (or cross) with $CO_2$ at 100 bar to form an emulsion.
- The emulsion is rapidly expanded to atmospheric pressure through flow restrictor to generate aerosols of microbubbles and microdroplets.
- The aerosol plume is dried at temperatures below 60 °C as it mixes with nitrogen or air in the drying chamber.
- Dry fine powders are collected and packaged.

# CAN-BD Mixing Tee

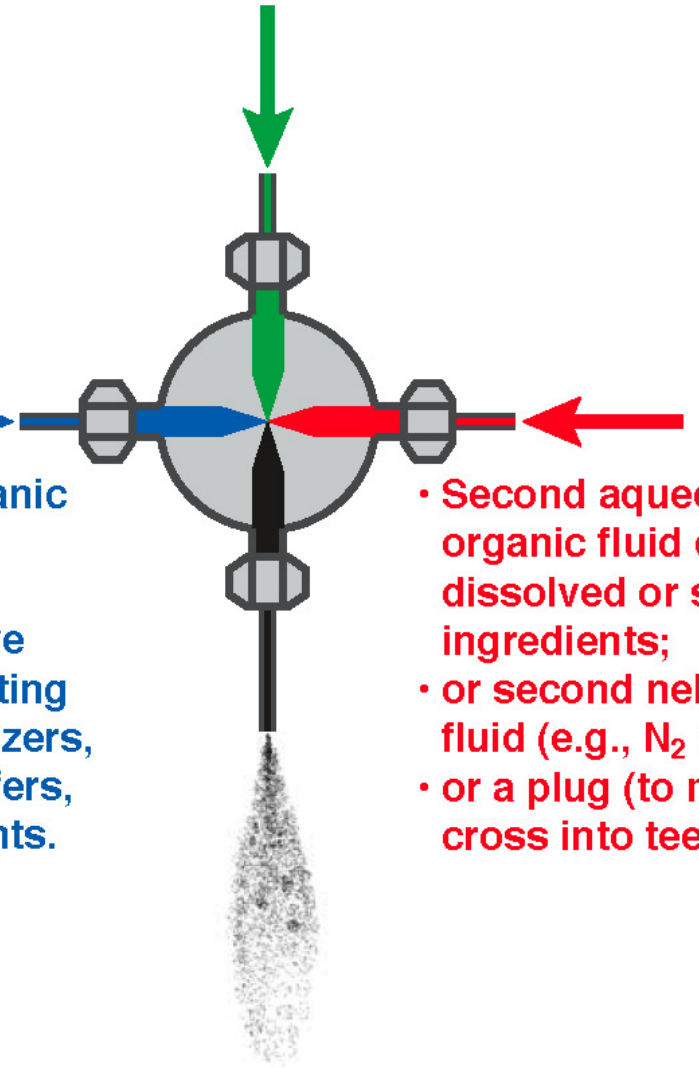
## Drying Temperature: 1°C to 60°C



# LOW DEAD VOLUME CROSS FOR CAN-BD

**Fluid 1**

Nebulizing supercritical or near-critical fluid (e.g., $CO_2$)

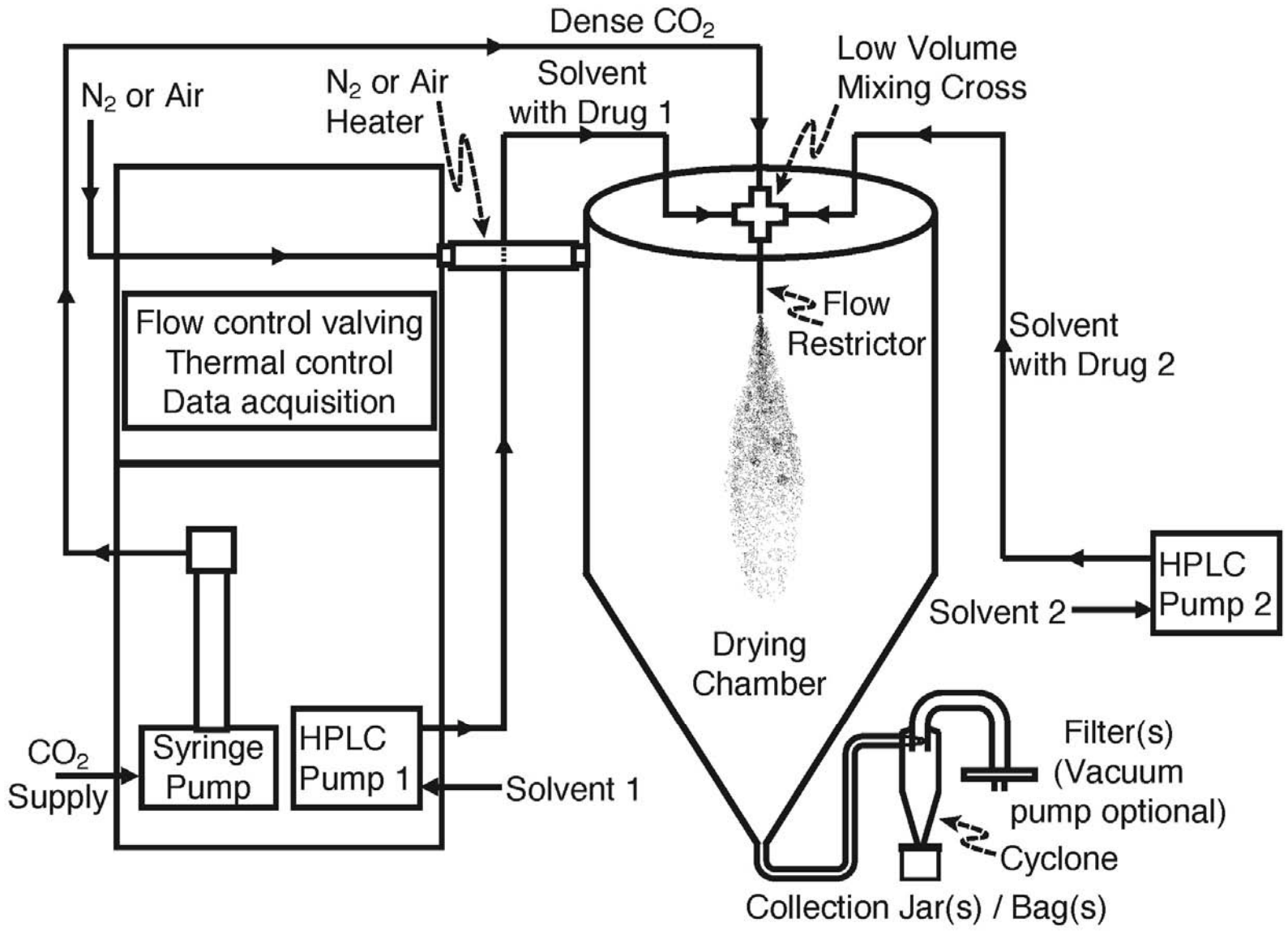


**Fluid 2**

- Aqueous or organic fluid containing dissolved or suspended active ingredients, coating materials, stabilizers, surfactants, buffers, or other excipients.

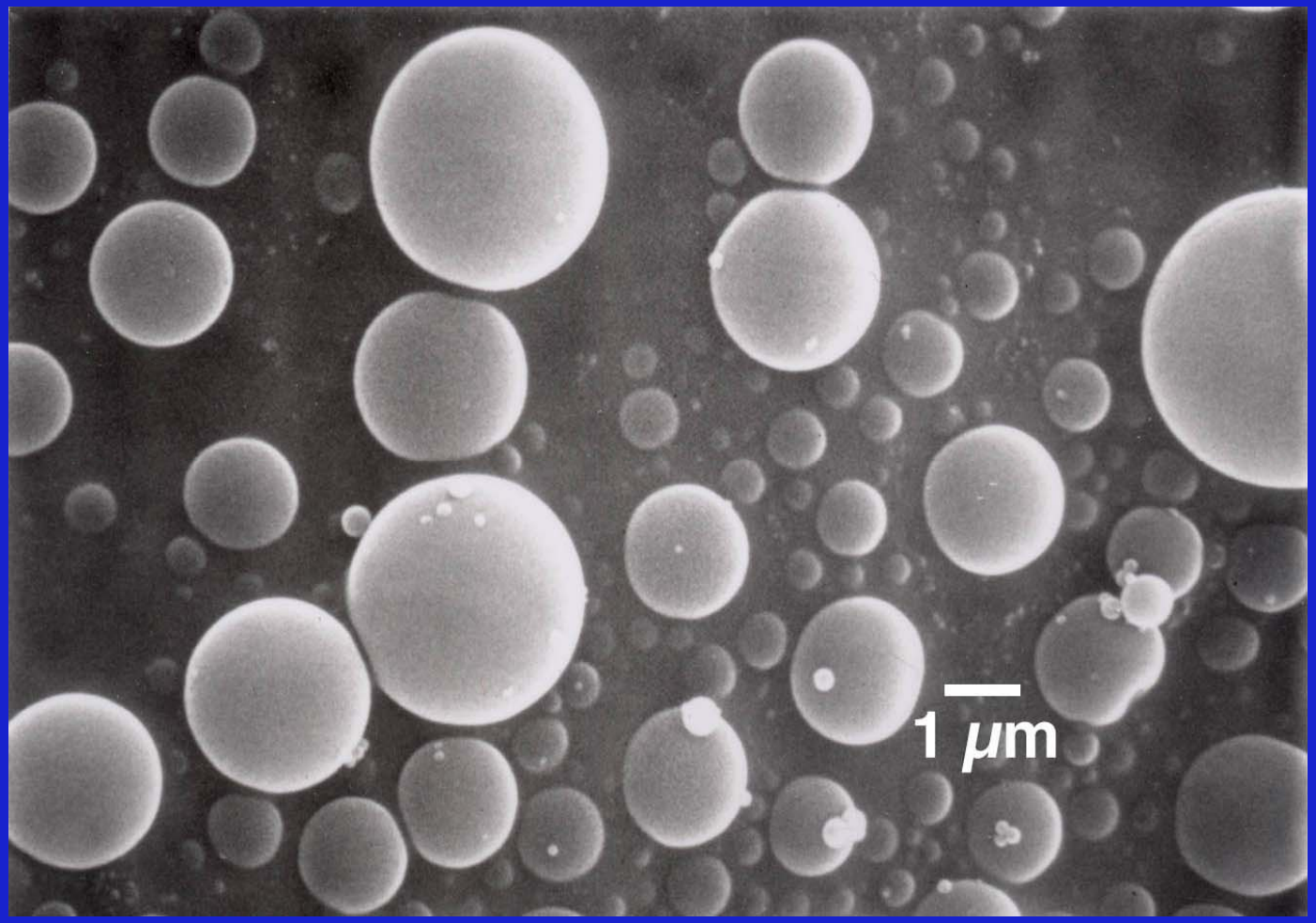
**Fluid 3**

- Second aqueous or organic fluid containing dissolved or suspended ingredients;
- or second nebulizing fluid (e.g., $N_2$ or air);
- or a plug (to make cross into tee)


Dense $CO_2$
$N_2$ or Air
$N_2$ or Air
Heater
Solvent
with Drug 1
Low Volume
Mixing Cross
Flow control valving
Thermal control
Data acquisition
Flow
Restrictor
Solvent
with Drug 2
HPLC
Pump 2
Solvent 2
Drying
Chamber
$CO_2$
Supply
Syringe
Pump
HPLC
Pump 1
Solvent 1
Filter(s)
(Vacuum
pump optional)
Cyclone
Collection Jar(s) / Bag(s)

**Aerosol spray of microbubbles and droplets generated by the CAN-BD process**

# LYSOZYME

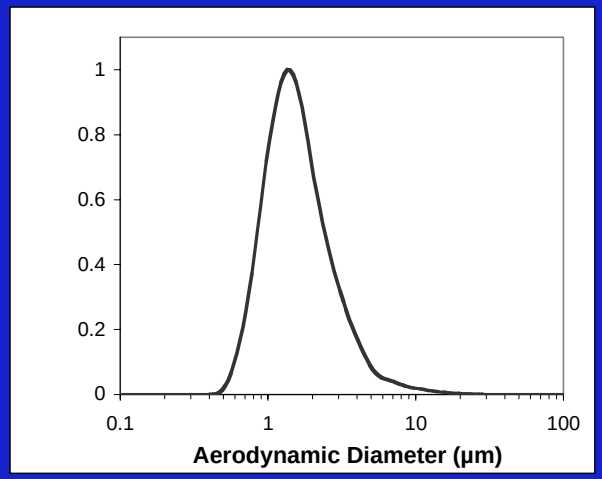


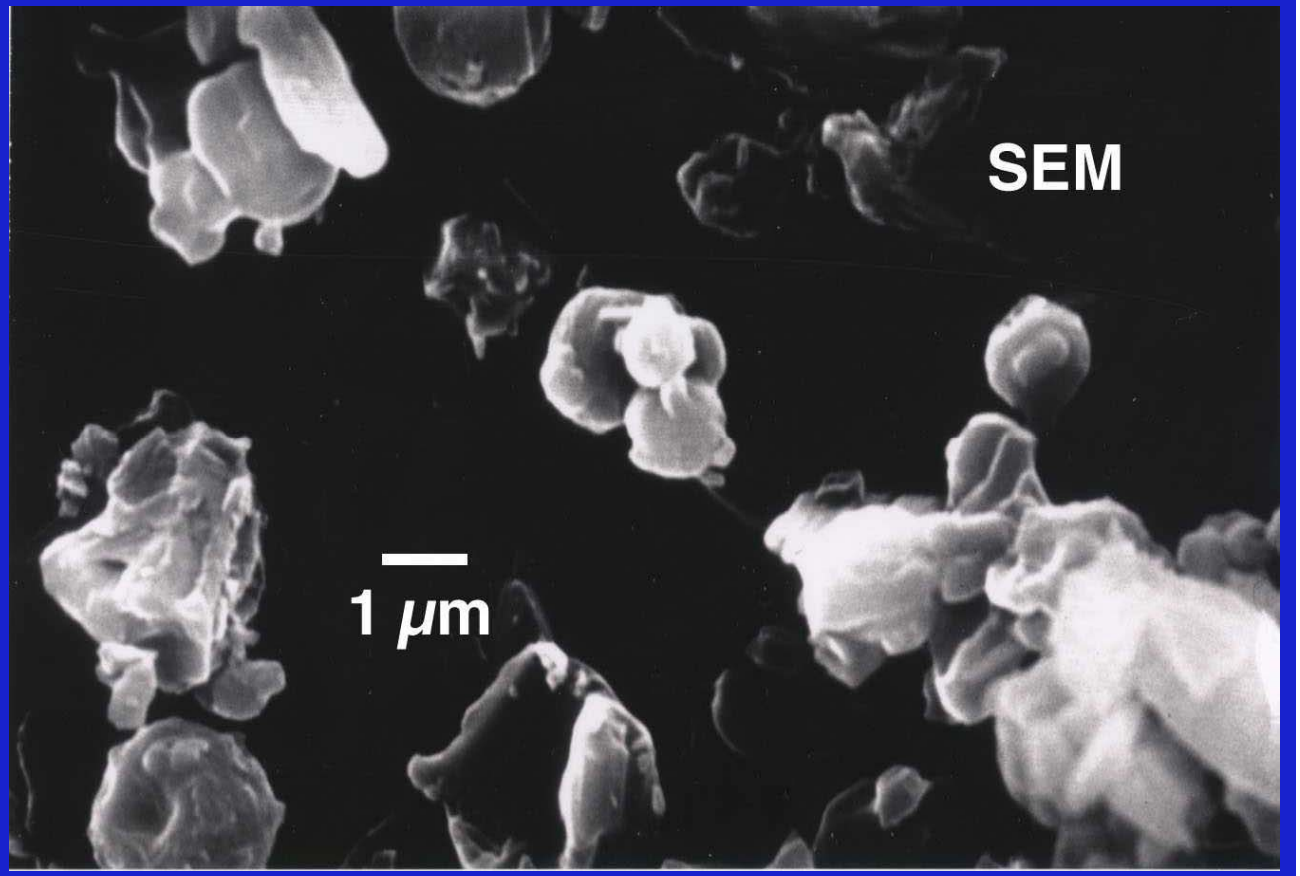

- Particles formed at 50 °C from an aqueous solution containing 10% lysozyme, 0.01% PEG 3350 and 0.01% Tween 80
- Mean aerodynamic diameter = 1.4 μm (95% < 3.0 μm)

# Aerosol Delivery of Antibiotics to Lungs

- Higher doses delivered locally to treat lung infections
- Reduced whole body burden of antibiotics
- Longer persistence in lung air space and tissue possible

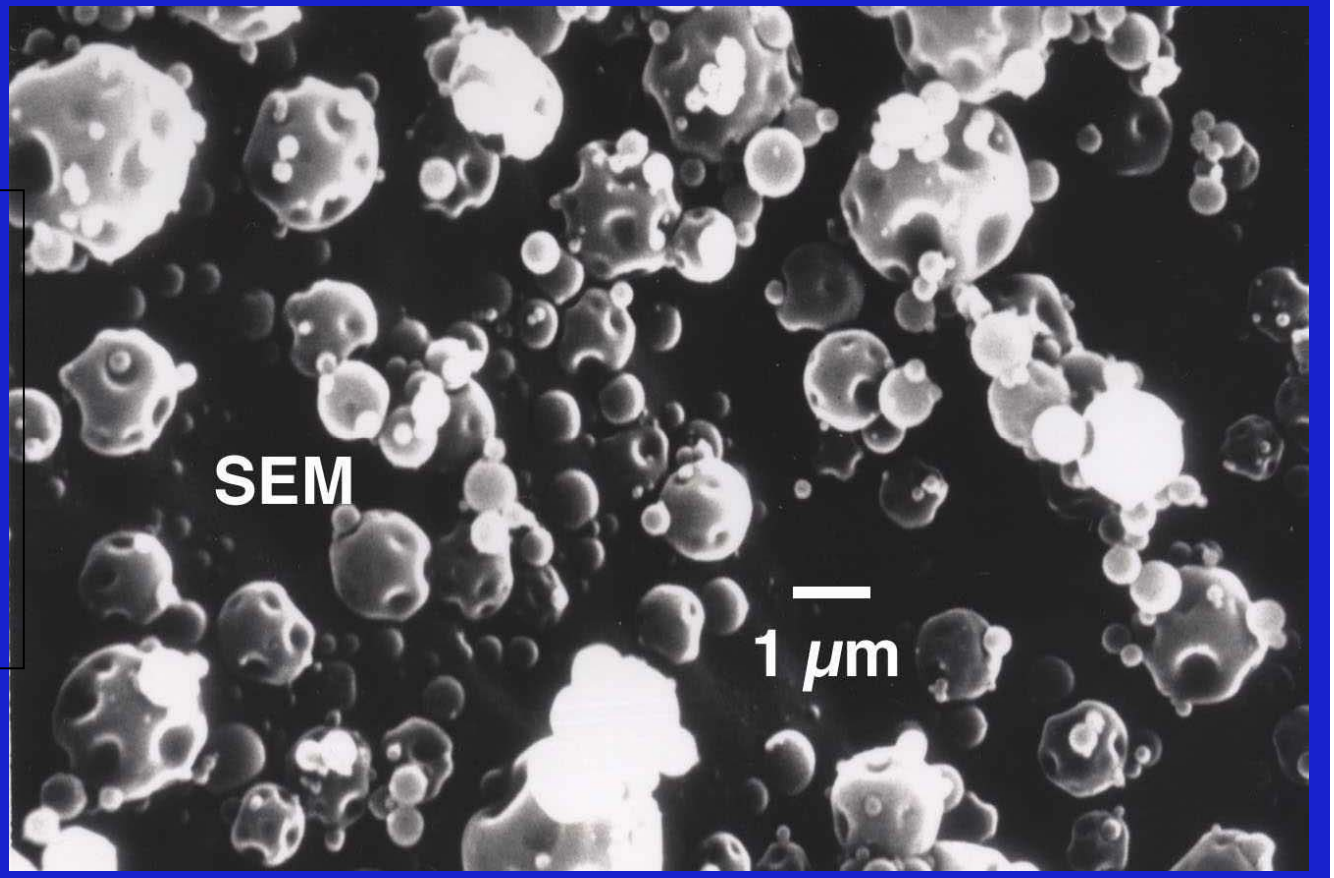
# Ciprofloxacin hydrochloride



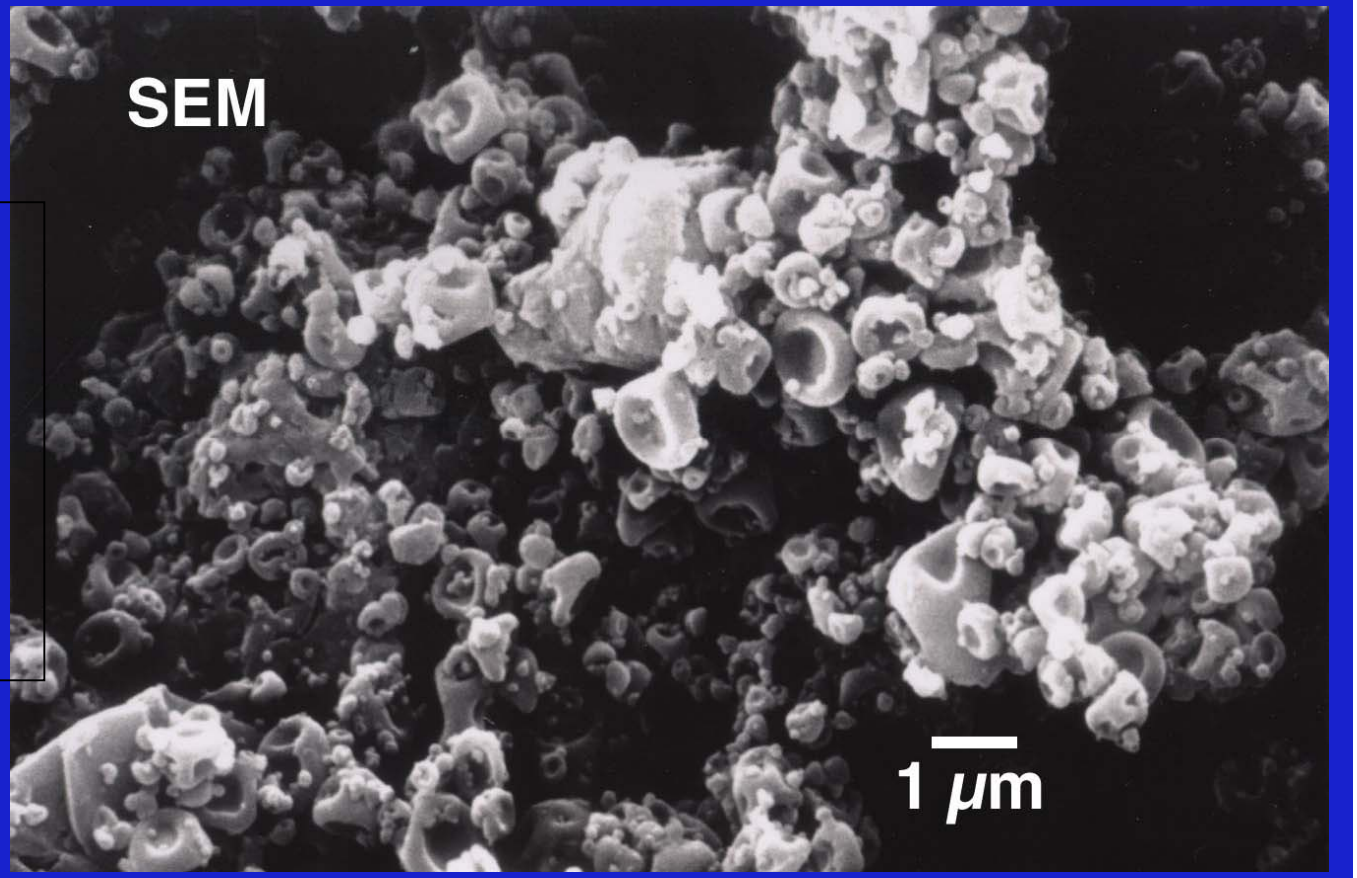

- Size distribution (by Aerosizer) and SEM of ciprofloxacin hydrochloride micronized by CAN-BD from a 3% aqueous solution. Dried at 60°C.
- Mean aerodynamic diameter = 1.6 µm; with 95% under 4.1 µm

# Moxifloxacin hydrochloride

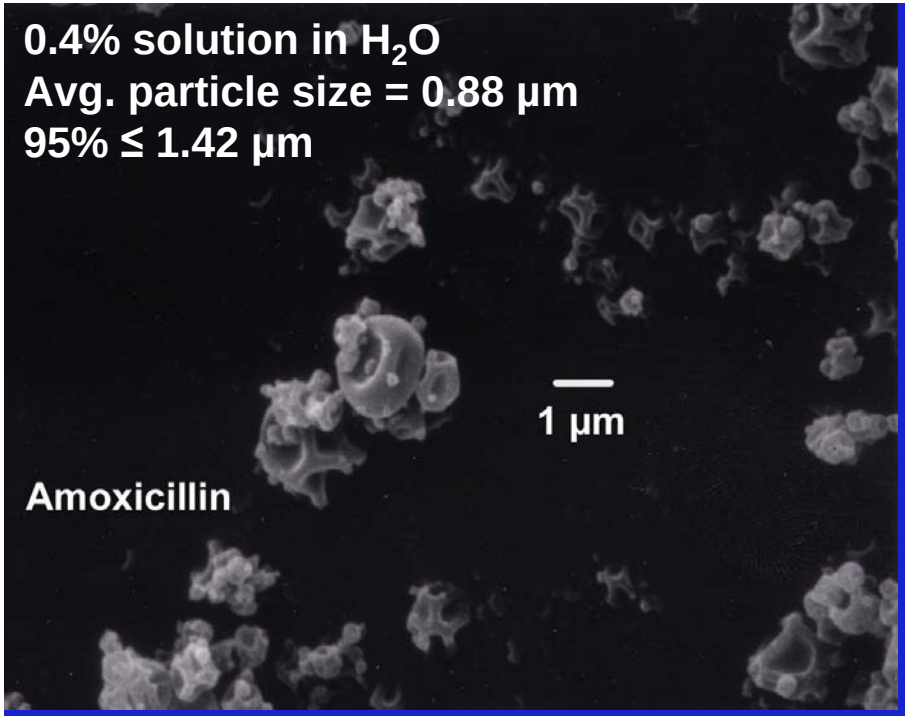


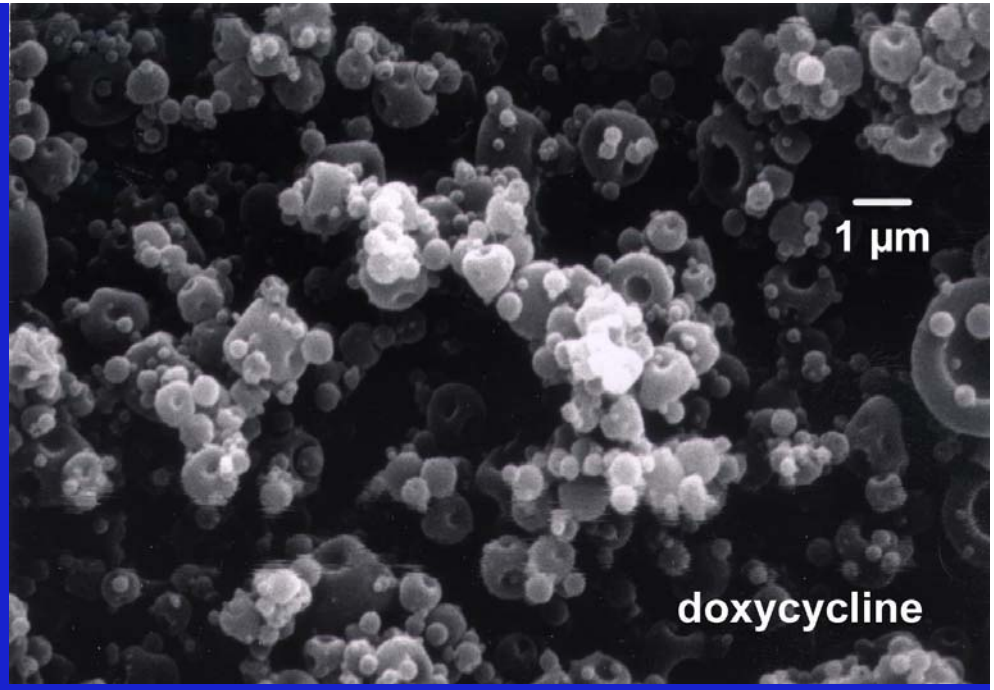

- Size distribution (by Aerosizer) and SEM of moxifloxacin hydrochloride micronized by CAN-BD from a 2% aqueous solution. Dried at 60°C.
- Mean aerodynamic diameter = 1.3 μm; with 95% under 2.5 μm

# Rifampicin

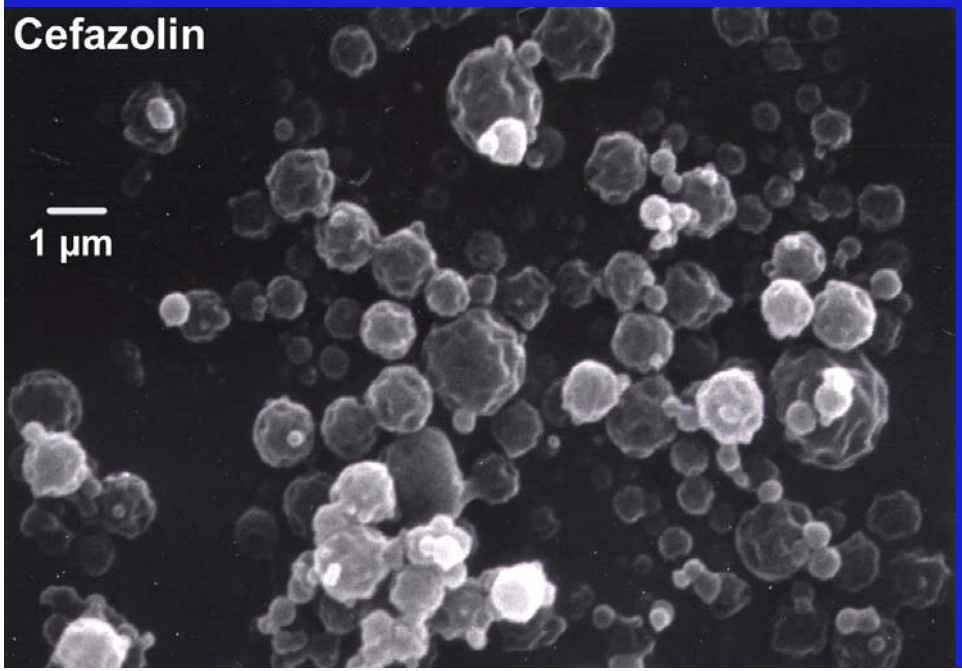



- Size distribution (by Aerosizer) and SEM of rifampicin micronized by CAN-BD from a 2% solution in ethyl acetate. Dried at 30°C.
- Mean aerodynamic diameter = 0.78 μm; with 95% under 1.3 μm




0.5% solution in $H_2O$
Avg. particle size = 0.97
95% ≤ 1.60 µm




# Particles of antibiotics generated from aqueous solutions

0.5% solution in $H_2O$
Avg. particle size = 1.05
95% ≤ 1.82 µm

*Mexico-INSP*

# Measles aerosol immunization

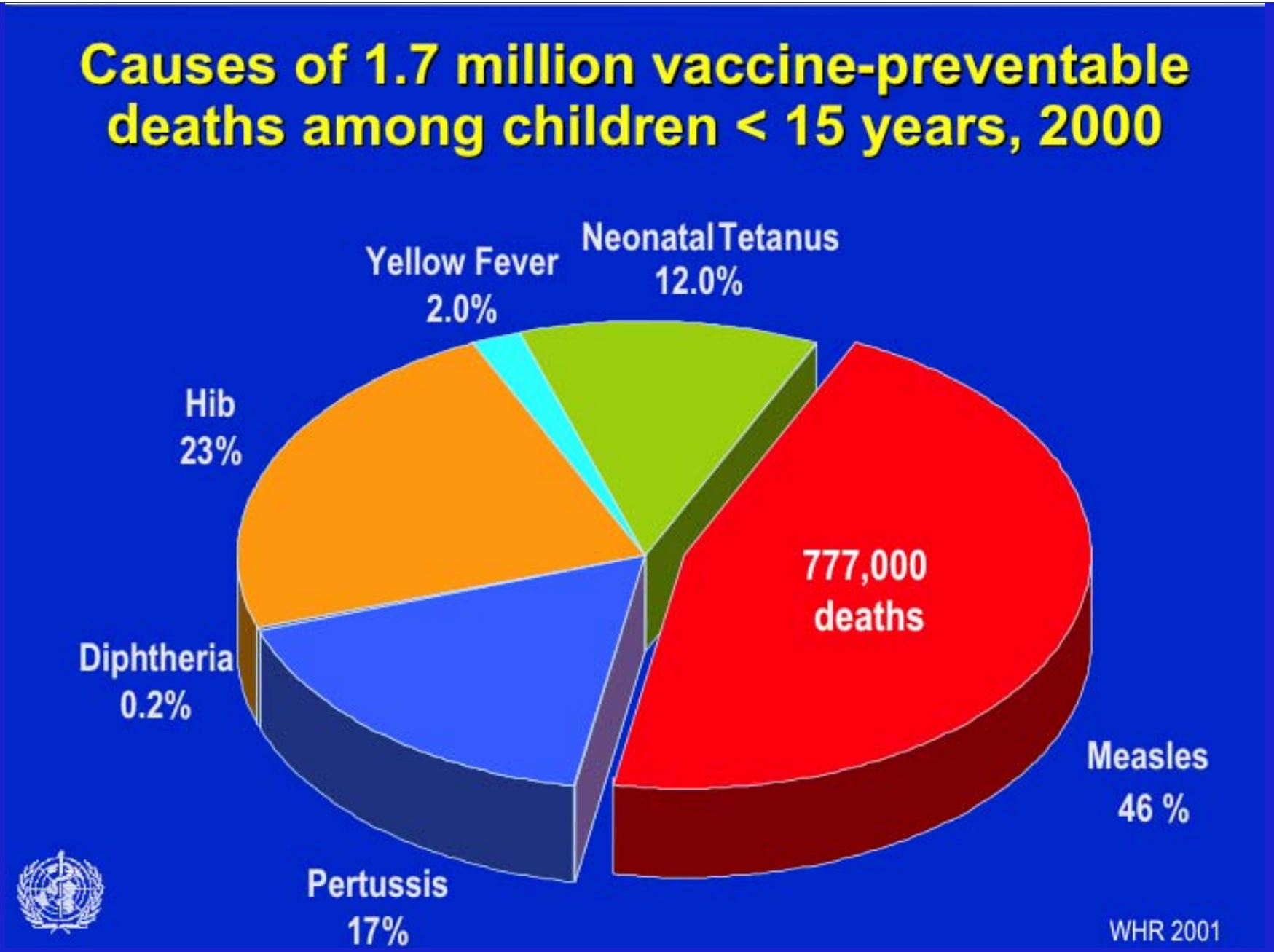
**SAFE** ☑ no serious AEFIs, fewer than SC route

**IMMUNOGENIC**
- ☑ induced >80% response among infants < 9 months of age
- ☑ 86-100% response in studies (1961-2002) among ≥ 9 months & school-aged children
- ☑ **good response with rubella vaccine**

**EFFECTIVE** ☑ lower attack rate (outbreak Mexico 1988-90):
- immunized with aerosol *(0.8%)*
- immunized with s-c *(14%)*
- unvaccinated group *(26%)*

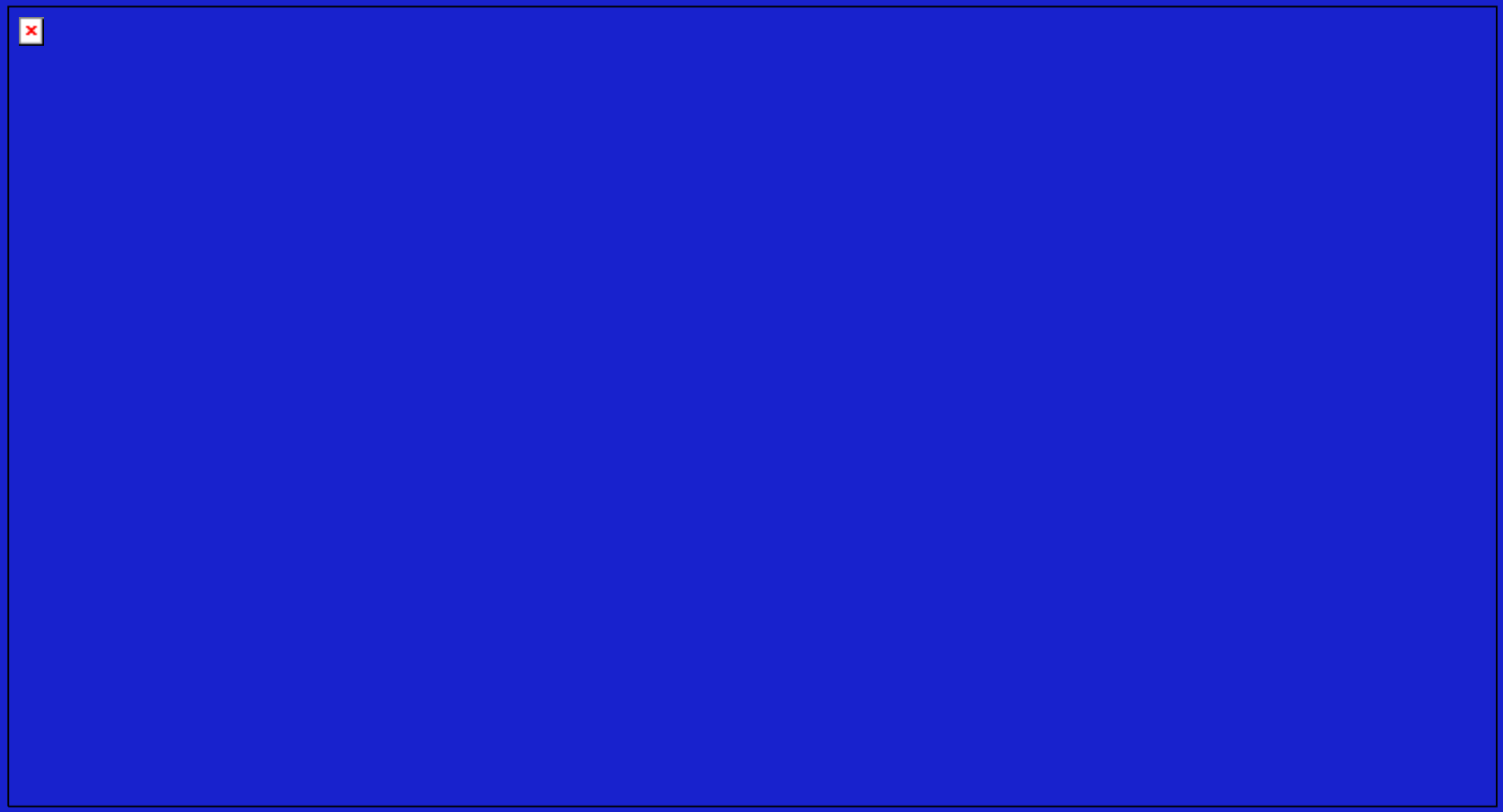
EZ strain retains potency during nebulization process

Henao-Restrepo and Papania, NIH Vaccines Conference, Rockville, Dec 2003




Henao, An Overview of Aerosol Immunization, Meeting of the WHO Steering Committee on New Delivery Systems, 2004

# CAN Processing of Live Virus Measles Vaccines



P. Rota, MD and M. Papania, MD, CDC, Atlanta, provided viral assays;
J. Burger, S. Cape, PhD, and E. Huang, PhD, A-D and CU, performed CAN-BD;
* B. Shekunov et al., Ferro, have achieved 34% yield by CAN-SFD.
Partial funding provided by Creare, Inc.

# Measles Vaccine Team

Aktiv-Dry
- Robert E. Sievers
- Brian Quinn
- John Carpenter
- Jessica Burger

Serum Institute of India
- Subhash V. Kapre
- R.M. Dhere

Becton Dickinson and Co.
- Vincent J. Sullivan

Centers for Disease Control & Prevention
- Mark J. Papania
- Paul Rota

Ferro Pfanstiehl
- Pratibhash Chattopadhyay
- Boris Shekunov

University of Colorado
- Stephen Cape
- Ed Huang
- Ted Randolph
- Shelly Miller
- Mark Hernandez

National Jewish Medical Research Ctr.
- Kevin Kisich

University of Kansas
- S. Russell Middaugh

University of Maryland
- Milagritos D. Tapia

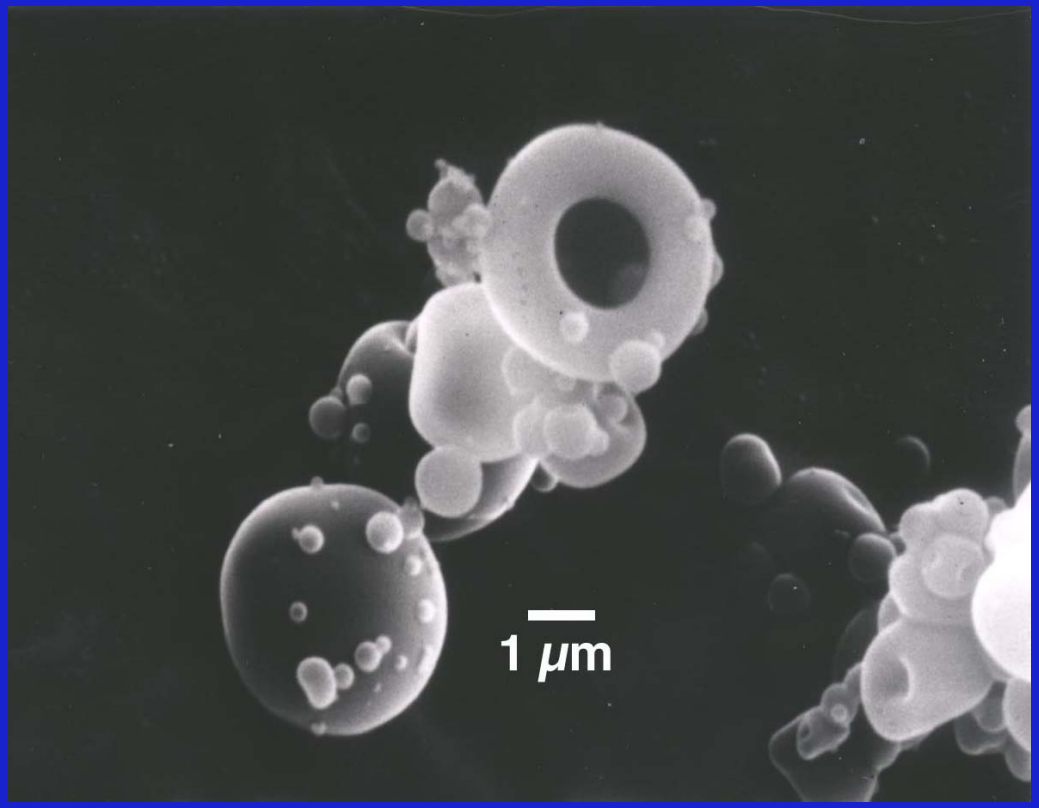
Johns Hopkins School of Medicine
- Beth L. Laube

CIT
- Roy Forster

ZymoGenetics, Inc.
- Eric Sievers

# Human Intravenous Immune Globulin (IVIG) Particles

**SEM Image**

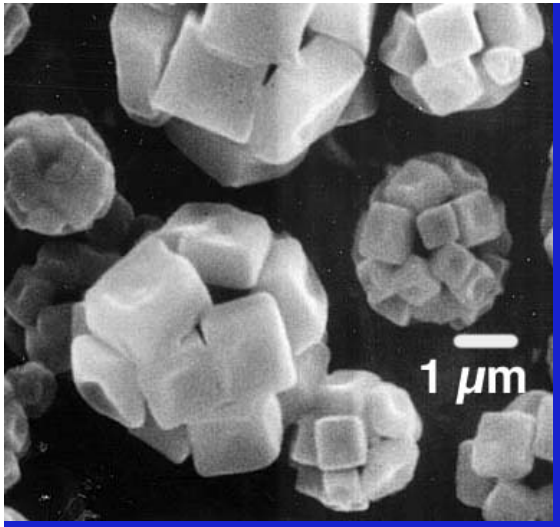



**Size Distribution**

- **Mean aerodynamic diameter = 1.45 μm**
- **95% of particles < 2.75 μm**

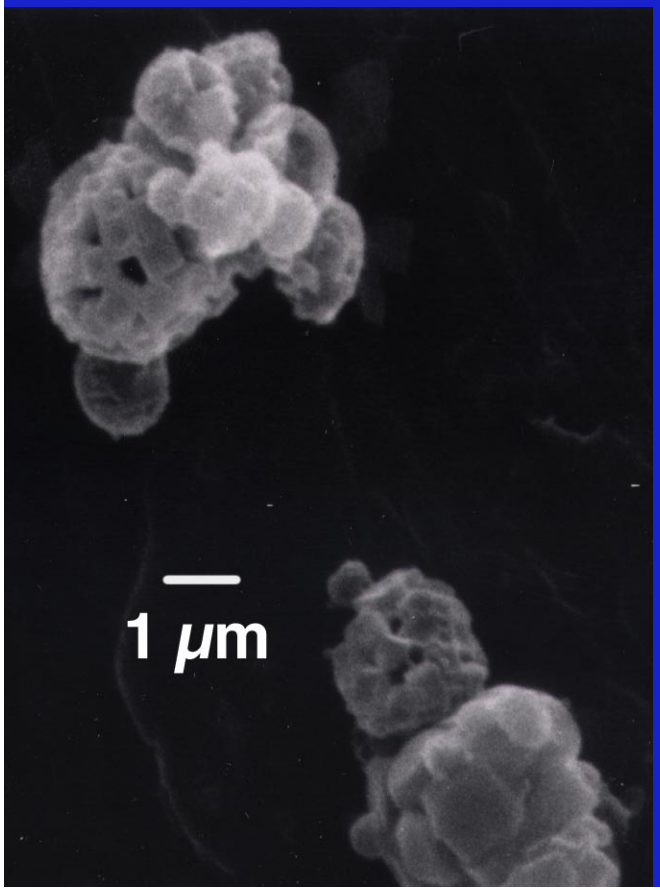
# SOLVENTS FOR CAN-BD

- Water
- Carbon Dioxide
- Methanol
- Ethanol
- Acetone
- Ethyl acetate
- Methylene chloride (only when required)
- Mixtures of solvents



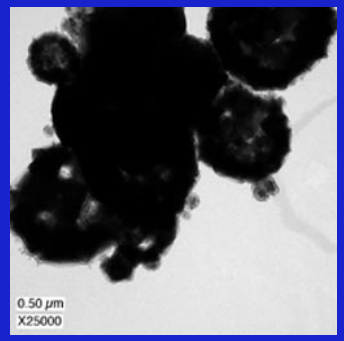

## Two Fluid Particle Formation (Tee)

SEM of particles generated from a 10% aqueous solution of NaCl with $CO_2$




## Three Fluid Particle Formation (Cross)

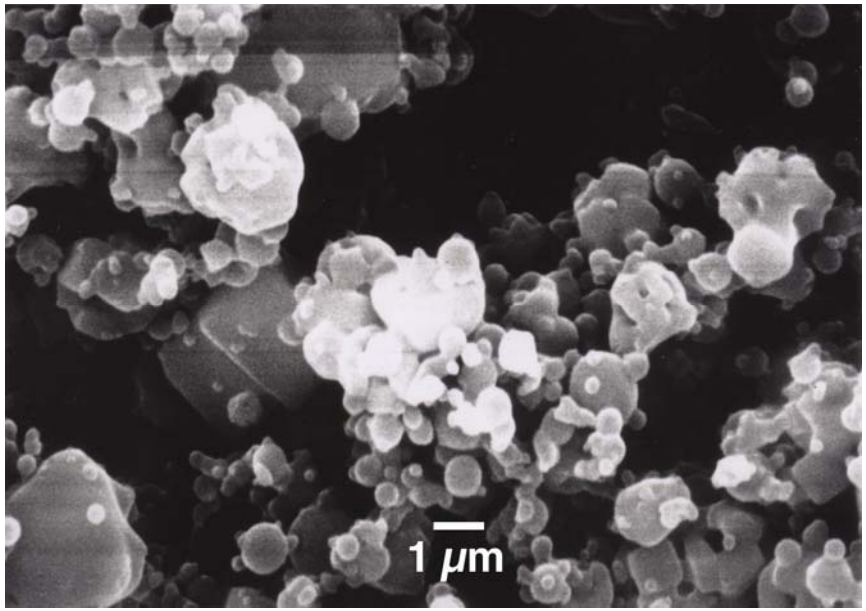
SEM/TEM of particles generated from a 10% aqueous solution of NaCl + 0.5% solution of PLGA in acetone with $CO_2$



# Coating of Particles by CAN-BD of Suspensions From a Tee

## Light Coating

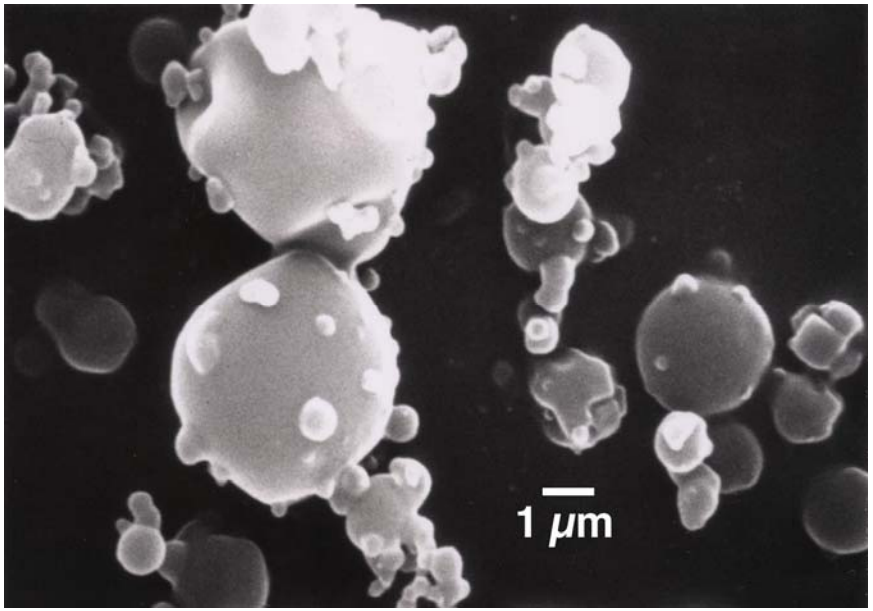
PLGA : NaCl (1:1)



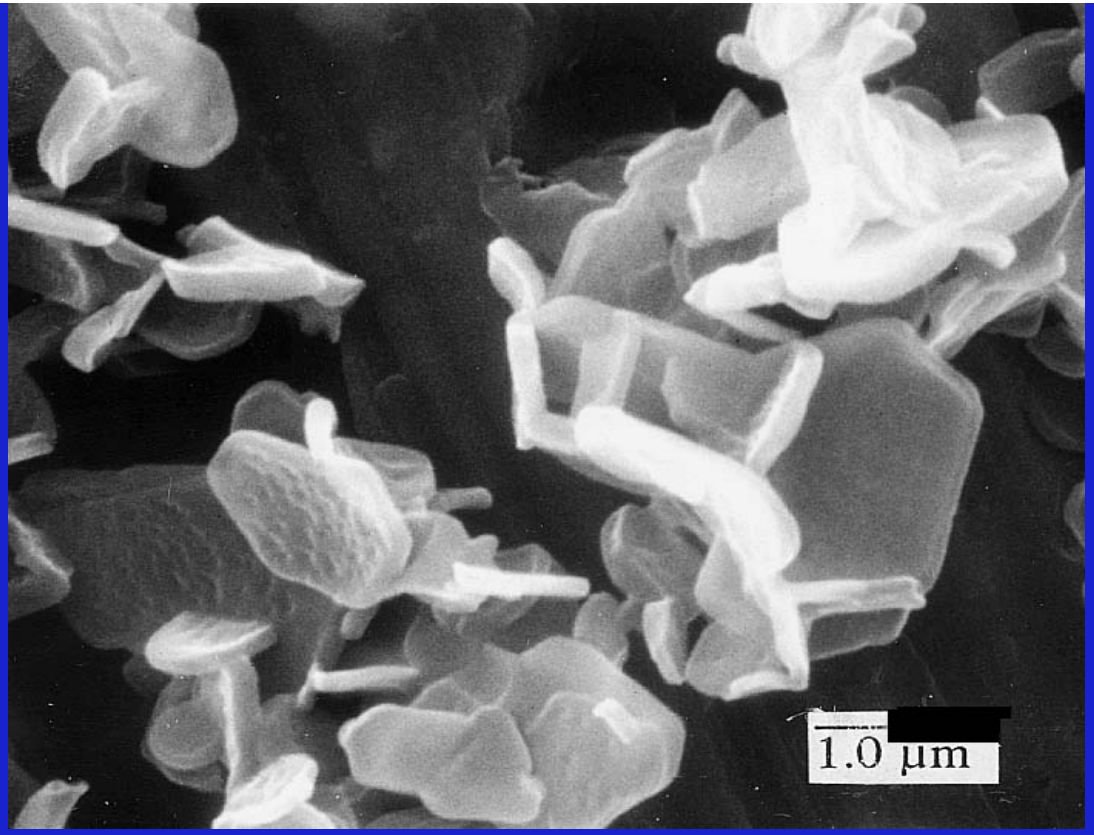

- **Coated particles from a suspension containing 0.5% NaCl particles and 0.5% dissolved PLGA**
- **Mean aerodynamic diameter = 1.8 μm (95% 4.2 μm)**

## Heavy Coating

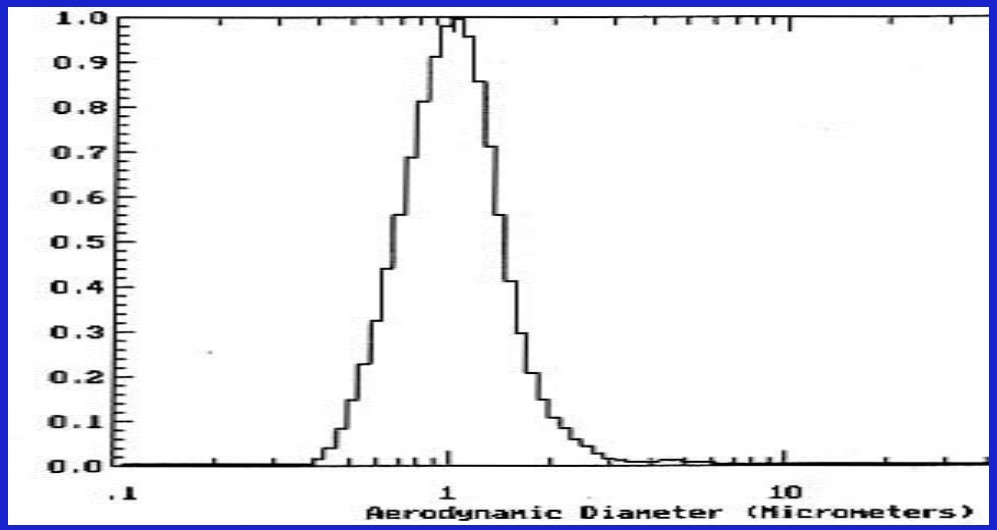
PLGA : NaCl (4:1)




- **Coated particles from a suspension containing 0.5% NaCl particles and 2% dissolved PLGA**
- **Mean aerodynamic diameter = 2.0 μm (95% 5.2 μm)**

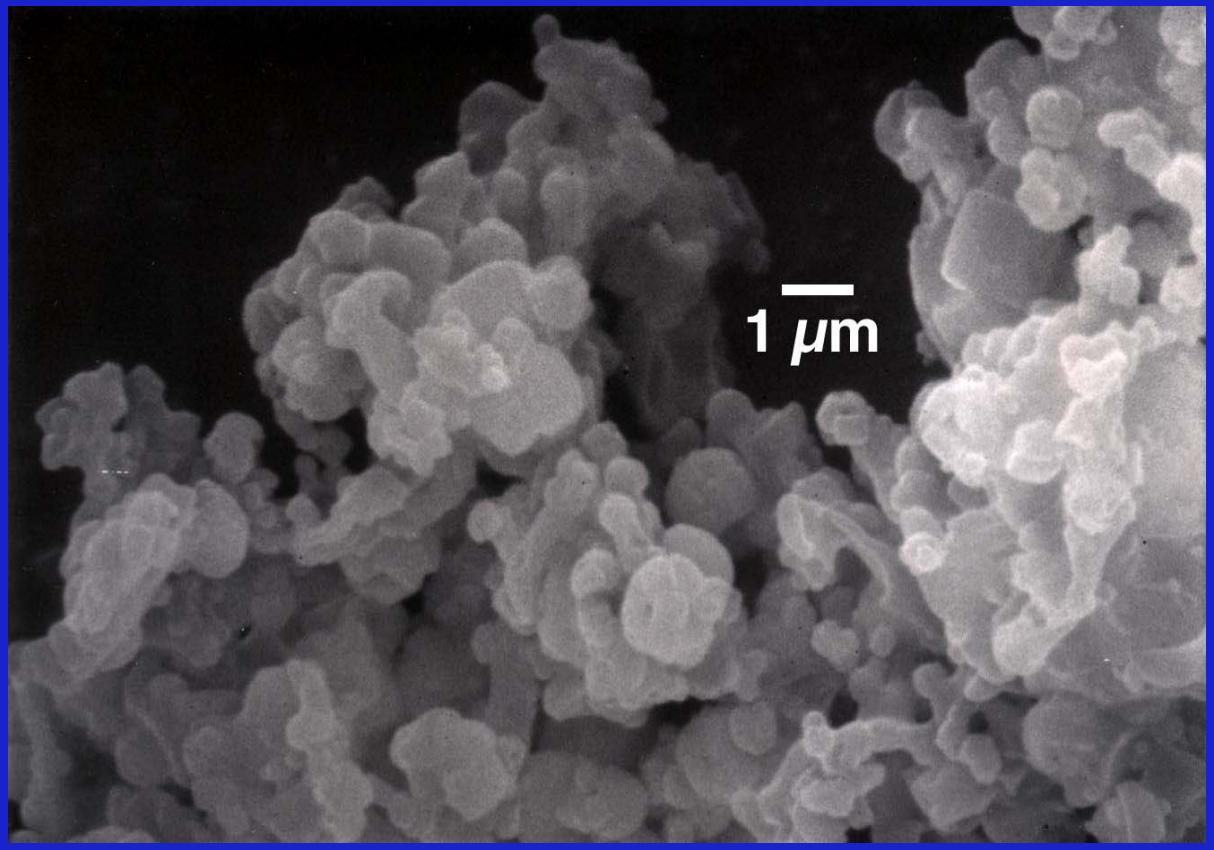



**SEM of crystalline palmitic acid particles generated by CAN-BD from an ethanolic solution containing 4% palmitic acid**



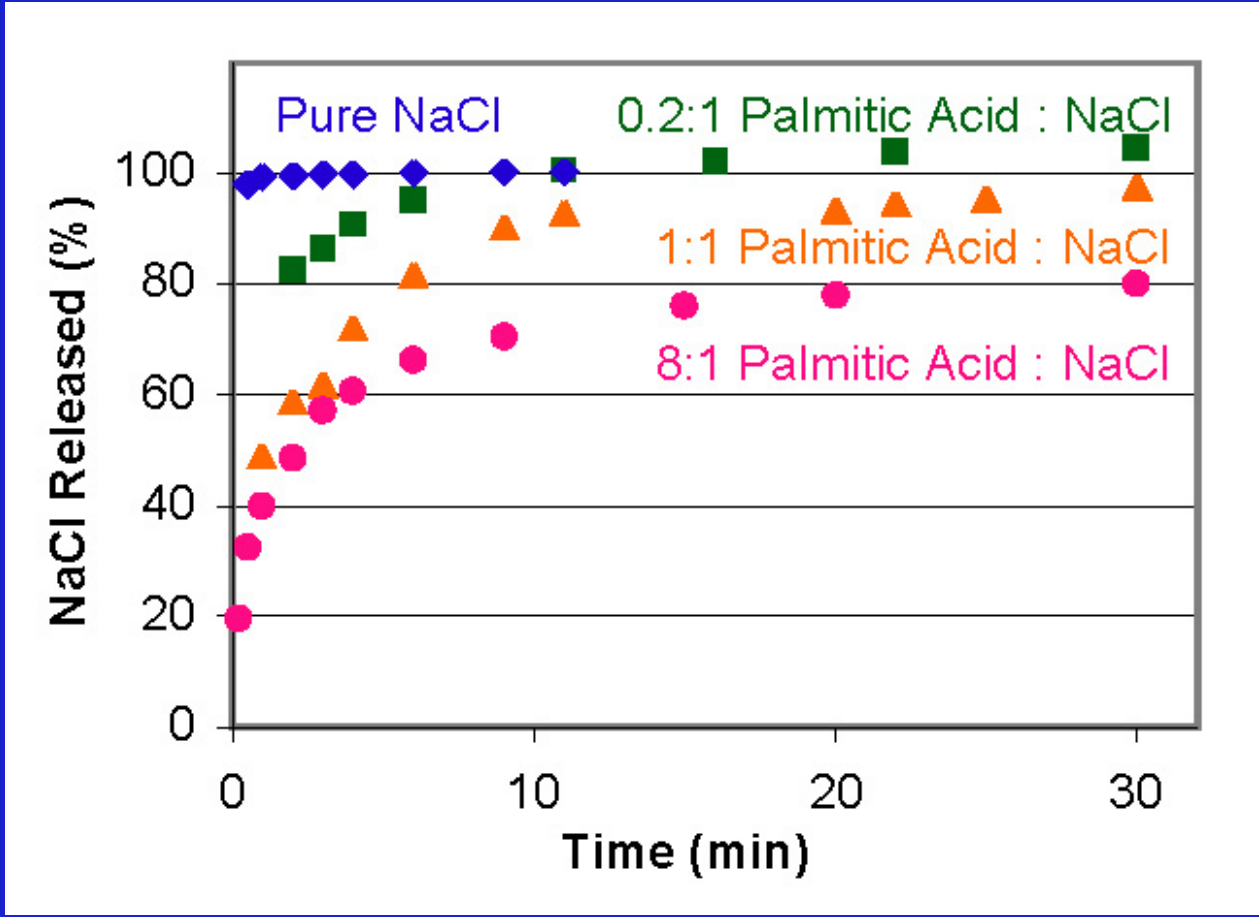

**average aerodynamic diameter**
**= 1.03 µm**
**99% < 3.40 µm**

# Heterogeneous Particles of Palmitic Acid Mixed with NaCl

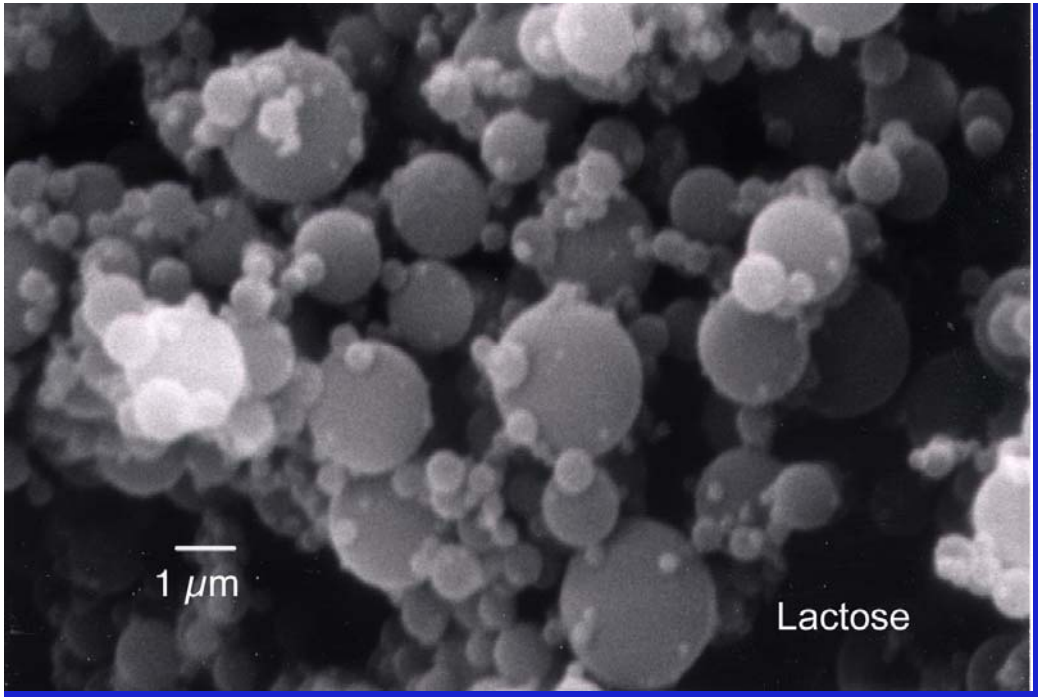



- Particles generated by CAN-BD from multiple fluids in a cross at 60 °C
  - 2% NaCl (water) and 2% Palmitic Acid (ethanol)
- 1 to 1 mass ratio of microcrystalline palmitic acid and NaCl formed
- Mean aerodynamic diameter = 1.5 μm (95% < 3.3 μm)

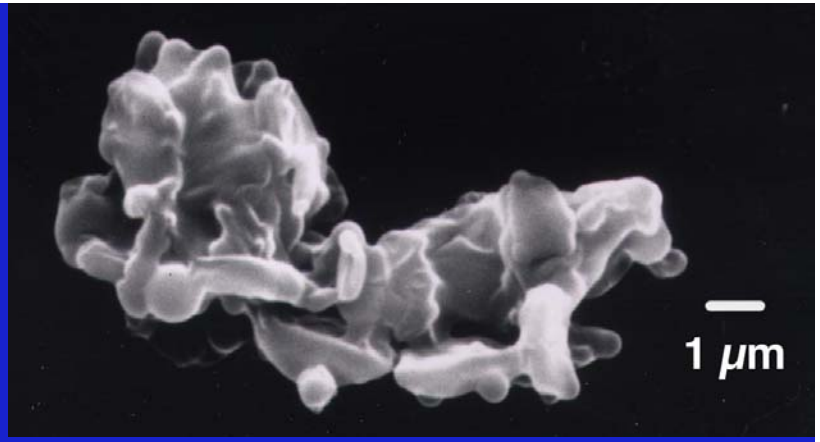
# In Vitro Controlled Release of NaCl from Heterogeneous Particles of Palmitic Acid Mixed with NaCl




**Particles generated by CAN-BD from ethanolic and aqueous solutions in a cross at 60 °C**



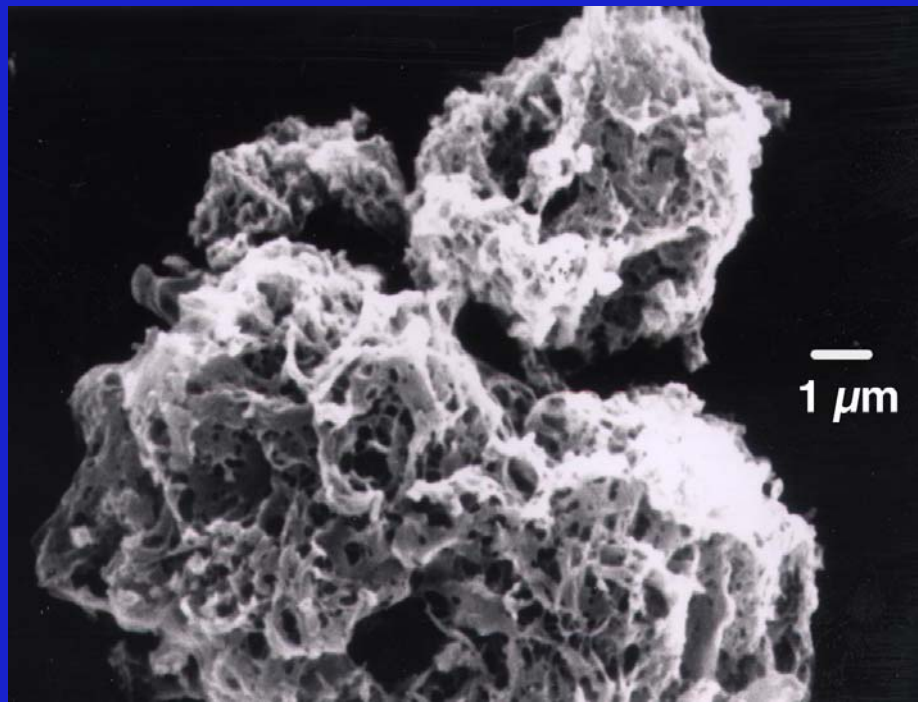

SEM of particles generated from a 10% aqueous solution of lactose with $CO_2$ in a TEE




SEM of particles generated from a 2% aqueous solution of lactose + 2% solution of palmitic acid in ethanol with $CO_2$ in a CROSS

## SELECTIVE LEACHING OF HETEROGENOUS PARTICLES

Particles suspended in EtOH, stirred for 0.5 hr, and filtered
Avg. aerodynamic diam. = 1.03 μm



# REPRESENTATIVE SOLUTES AND SUSPENSIONS THAT HAVE BEEN MICRONIZED BY SUPERCRITICAL PROCESSING

| | |
|---|---|
| **Vaccines:** | **attenuated live measles virus vaccine, live B/Harbin influenza virus vaccine** |
| **Antibodies:** | **anti-CD4, IVIG, anti-human lambda light chain** |
| **Enzymes:** | **$\alpha_1$-antitrypsin, trypsinogen, lysozyme, lactate dehydrogenase** |
| **Antibiotics:** | **ciprofloxacin, moxifloxacin, rifampicin, isoniazid, tobramycin sulfate, amoxycillin, doxycycline** |
| **Water-soluble drugs:** | **albuterol sulfate, cromolyn sodium** |
| **Alcohol-soluble drugs:** | **naproxen, budesonide, betamethsone, amphotericin B, cyclosporin, DPPC** |
| **Sugars:** | **lactose, sucrose, trehalose, mannitol** |
| **Polymers:** | **PLA, PLGA, PEG** |
| **Amino acids:** | **methionine, arginine** |

# Conclusions

- Multiple constituents can be incorporated in three fluids in a low volume cross to make heterogenous and homogenous nanoparticles and microparticles.
- Pharmaceuticals, proteins, antibodies, and vaccines can be stabilized, dried, and micronized by a newly patented process, CAN-BD.
- A SF nebulization, desolvation and coating method (CAN-BD) has been developed that can manufacture dry powders and coatings of many materials.
- Coating and drying requires only seconds.
- Water and many organic solvents yield nanoparticles and microparticles with unique compositions and properties.
- Fluid ratios, pressures, and solute concentrations determine particle size.
- For more data & applications, visit www.AKTIV-DRY.com